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The Iberian lynx *Lynx pardinus* Conservation Breeding Program

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The Iberian Lynx Conservation Breeding Program follows a multidisciplinary approach, integrated within the National Strategy for the Conservation of the Iberian lynx, which is carried out in cooperation with national, regional and international institutions. The main goals of the *ex situ* conservation programme are to: (1) maintain a genetically and demographically managed captive population; (2) create new Iberian lynx *Lynx pardinus* free-ranging populations through re-introduction. To achieve the first goal, the Conservation Breeding Program aims to maintain 85% of the genetic diversity presently found in the wild for the next 30 years. This requires developing and maintaining 60–70 Iberian lynx as breeding stock. Growth projections indicate that the *ex situ* programme should achieve such a population target by the year 2010. Once this goal is reached, re-introduction efforts could begin. Thus, current *ex situ* efforts focus on producing psychologically and physically sound captive-born individuals. To achieve this goal, we use management and research techniques that rely on multidisciplinary input and knowledge generated on species' life history, behaviour, nutrition, veterinary and health aspects, genetics, reproductive physiology, endocrinology and ecology. Particularly important is adapting our husbandry schemes based on research data to promote natural behaviours in captivity (hunting, territoriality, social interactions) and a stress-free environment that is conducive to natural reproduction.

Key-words: adaptive management; applied research; conservation breeding; *ex situ*; genetics; husbandry; Iberian lynx; outreach; re-introduction; reproductive physiology; veterinary science.

INTRODUCTION

Iberian lynx *Lynx pardinus* wild populations have undergone a constant regression throughout the last century. The decline has been especially abrupt in the last 20 years, with more than an 80% reduction, mostly owing to the dramatic decline of wild rabbit *Oryctolagus cuniculus* populations, the lynx's prey base. According to the last census (Guzmán *et al*., 2002), <200 Iberian lynx (only half of them considered to be adults with reproductive potential) survive in nature. The last two remnant populations, Doñana and Sierra Morena, are located in Andalusia, southern Spain. This dramatic population decline has brought the species to what is known as an 'extinction vortex'. The small size of both populations makes them highly vulnerable to stochastic events, such as natural disasters (e.g. forest fires, flooding), disease outbreaks, genetic and demographic problems, etc., that could completely wipe out a remnant population within a very short period of time (Delibes *et al*., 2000). The species is listed as Critically Endangered by The World Conservation Union (IUCN, 2006).

Owing to the precarious situation of the Iberian lynx in the wild, conservation measures need to be implemented effectively and efficiently, integrating efforts and working tools (Heredia *et al*., 1999; MIMAM, 1999). Iberian lynx conservation could be conceived as a puzzle whose pieces should fit together adequately. Primary efforts should be directed towards *in situ* conservation, which includes (1) maintaining and expanding remnant populations, monitoring, managing prey (wild Rabbit) populations, protecting and restoring habitat, promoting agreements with land owners, minimizing non-natural causes of mortality and connecting, from a genetic standpoint, the two remnant populations, and (2) preparing habitat for creating new free-ranging populations. Another piece of the puzzle is *ex situ* conservation, which includes, among other activities, captive breeding, preparing animals for release, research in different areas, management of a Biological Resource Bank (BRB), genetic management of all lynx populations (wild and captive) as a single metapopulation, as well as training staff, education and outreach efforts (Vargas, Sánchez *et al*., 2005).

Current Iberian lynx conservation-breeding efforts focus on producing psychologically and physically sound captive-born individuals. For this purpose, we use management and research techniques that rely on multidisciplinary input and knowledge, generated on the life history of the species, behaviour, nutrition, veterinary and health aspects, genetics, reproductive physiology, endocrinology and ecology. Particularly important is adapting our husbandry schemes based on research data to promote natural behaviours in captivity (hunting, territoriality, social interactions) and a stress-free environment that is conducive to natural reproduction. Some relevant research areas include: determining faecal hormone profiles for adult and subadult lynxes, studying reproductive behaviour and cub development, determining the reproductive health of ♂ and ♀ breeders, developing a non-invasive pregnancy test, establishing sound biosecurity and biomedical protocols, genotyping all founders and make pairing recommendations based on genetic distance between breeders, etc. One of our goals is to minimize the use of potentially invasive methods while simultaneously enhancing the trust between the lynxes and their keepers to assist in securing information on animal body mass and gestational status. Over the past 3 years, eight pregnancies have resulted in the birth of 19 offspring, of which 11 survive to date (Vargas *et al*., 2007). While describing various organizational aspects of the Iberian lynx Conservation Breeding Program, this paper will also emphasize how results from multidisciplinary life-science research can be integrated into an adaptive management approach to help recover the world's most threatened felid species.

THE IBERIAN LYNX *EX SITU* CONSERVATION PROGRAM

The Iberian Lynx *Ex Situ* Conservation Breeding Program follows a multidisciplinary approach, integrated within the National Strategy for the Conservation of the Iberian lynx, officially endorsed by the Spanish National Commission for the Protection of Nature. National, regional and international institutions collaborate with the Program, which is currently implemented through a 'multilateral commission' that involves the central governments of Spain and Portugal, together with the autonomous governments of Andalusia, Extremadura and Castilla-La Mancha, Spain. Portugal, where no Iberian lynx populations were detected during the last 2002–2003 census, has developed its own *ex situ* conservation action plan, prepared in coordination with the Iberian lynx captive breeding committee (Serra *et al*., 2005; see also Vargas, 2006).

The main goals of the Iberian Lynx Conservation Breeding Program are twofold: (1) to maintain a genetically and demographically managed captive population that serves as a 'safety net' for the species and (2) to help establish new Iberian lynx free-ranging populations through re-introduction programmes. Because the extraction of large

number of lynxes from the last two remnant populations could compromise their viability, the most viable way to create new populations is through the re-introduction of individuals born in captivity. To achieve the latter goal, the Conservation Breeding Program must be integrated with *in situ* measures, such as the conservation and restoration of potential Iberian lynx habitat in areas of historical presence of the species (Andalusia, Castilla-La Mancha, Extremadura and Portugal)

The Iberian Lynx Program encompasses management and applied research strategies in the following six areas.

1. *Genetic and demographic management of the captive population* The best genetic management of threatened captive populations is achieved through rapid population growth until the established number of individuals required to maintain genetic variability for the species in question is reached (Lacy, 1994). Afterwards, population size should be stabilized. In order to implement this approach, the production of new individuals must be planned properly to meet both re-introduction and breeding programme needs, because the latter will gradually require the replacement of older individuals past their reproductive prime. Genetic and population management must be accompanied by proper husbandry, which involves stimulating natural behaviours in captive-born individuals from early developmental periods in order to improve their potential for survival in the wild (Plate 1).

Plate 1. Iberian lynx *Lynx pardinus* ♀ **and cub which are part of the Iberian Lynx Conservation Breeding Program in Spain.** ***Iberian Lynx*** Ex Situ ***Conservation Program, Ministry of the Environment, Madrid, and Environmental Counsel of Andalusia (MMA-CMA).***

Based on recommendations provided by the IUCN Conservation Breeding Specialist Group, in collaboration with the Iberian lynx *in situ* conservation managers, the actual situation of this species will allow for the conservation of 85% of the current genetic variability for a period of 30 years (Lacy & Vargas, 2004; Godoy, 2006). Captive populations that maintain $< 85\%$ genetic variability are considered to be dangerously inbred.

In order to achieve the established genetic goals, four wild-born cubs/juveniles (founders) must be incorporated into the Breeding Program each year for 5 years consecutively. Thus, 20 cubs/juveniles are needed within a 5 year period. Wild-born founders should be selected from large litters (of three or more siblings), because the extraction of such individuals will have a lower impact in the wild population (Lacy & Vargas, 2004). The maintenance of genetic diversity over 30 years will, therefore, require a group of 60 (30.30) breeding animals (comprising the original founders plus individuals born in the Conservation Breeding Program). As a basic strategy for maintaining genetic variability, it is important to achieve rapid population growth over the first 10 years of the Program, until it reaches its 'capacity phase', established at 60 breeding individuals. Efforts should also be made to ensure equal representation of founders, which should all

provide a similar number of offspring to the Program.

2. *Captive husbandry* Captive husbandry is based on multidisciplinary input from a variety of animal-care fields, such as nutrition, behaviour, genetics, physiology and veterinary medicine, together with the systematic use of the scientific method. Over the past two decades, a great deal of knowledge and experience has been gained in the management of wild felids in captivity. The Association of Zoos and Aquariums Felid Taxon Advisory Group has compiled a *Husbandry Manual for Small Felids* containing useful information on, for example, health, reproduction, nutrition and facilities (Mellen & Wildt, 1998). Many European zoos have broad-ranging experience in breeding wild cats in general and lynxes in particular. These documents and experiences have been and continue to be a very useful reference to the Iberian Lynx Captive Breeding Program (Vargas *et al*., 2006).

One of the Program's key husbandry challenges is to strike a balance between fostering natural behaviours in captivity (hunting, territoriality, social interactions, etc.) and creating a stress-free environment where animals are more prone to mate. In order to obtain important information about the animals (such as their body mass or determining whether or not the ♀♀ are pregnant), certain training techniques are being used. Some of these include obtaining regular body-mass measurements by encouraging the lynx to step onto a measuring scale. Such techniques are designed to avoid using invasive methods, which would stress the animals, and they also serve as a way to strengthen the bond between the animals and their keepers.

The animals' behaviour is also being carefully observed by a round-the-clock video-surveillance system, which provides a great deal of information on the species that could not be learned easily through observations in the wild. Based on the experience acquired at the 'El Acebuche' Center and at the Jerez Zoo, together with information obtained from programmes established at European and American zoos, the El Acebuche Breeding Center has developed a standard operational-procedures manual that details the various protocols that are applied to its breeding population (see Vargas, Martínez *et al*., 2005). Detailed protocols based on experiences at the 'pilot facilities' will help smooth the way towards unified practices across *ex situ* lynx breeding centres as new centres open.

3. *Health and veterinary aspects* The health considerations involved in captive breeding, reintroduction and translocation programmes are a source of great concern to conservation biologists. For instance, mycoplasmosis in Mohave desert tortoises *Xerobates agassizi* (Jacobson, 1991), equine encephalitis in Whooping cranes *Grus americana* (Dein *et al*., 1986), diaphragmatic hernias in Golden lion tamarins *Leontopithecus rosalia* (Bush *et al*., 1993) and herpes virus in a large list of captive-bred avian species (Viggers *et al*., 1993) are just a few examples of health problems associated with small, threatened populations. There have been various cases in which captive-bred animals re-introduced into the wild have transmitted infectious diseases to wild populations; for example, tuberculosis in reintroduced Arabian oryx *Oryx leucoryx* (Viggers *et al*., 1993). Also, wild-caught individuals have occasionally infected captive populations with potentially lethal diseases [e.g. canine distemper in Black-footed ferrets *Mustela nigripes* (Williams *et al*., 1988)]. It is generally felt that most of these conservation programmes were lacking sufficient information on: (1) disease distribution and risk in captive populations; (2) disease incidence, distribution and risk in wild populations; (3) quarantine systems to prevent disease transmission; (4) a system to track and detect pathogens adequately.

Because relatively little is known about the diseases affecting lynx, actions to improve our knowledge of the main diseases affecting the species are imperative. The Iberian Lynx Conservation Breeding Program has a Veterinary Advisory Team dedicated to diverse aspects of veterinary and research management, as well as protocol development. To tackle the understanding of the various diseases that potentially affect the species, the

Program's main lines of action involve the establishment of preventive disease protocols for the captive populations, and research on general veterinary science (Martínez, 2006).

Projects are now under way to determine the incidence and prevalence of infectious pathogens in captive and wild lynx populations (Meli *et al*., 2006; Millán, 2006), determination of normal *vs* pathological blood values (Muñoz *et al*., 2006; Pastor *et al*., 2006) and research on potential renal disfunction (Jiménez *et al*., 2006). The results of research, protocol developments and standardization efforts, coupled with dissemination and sharing of knowledge and experience among veterinarians working in the programme, are all contributing to more consistent diagnosis and treatment. For further information, see http://www.lynxexsitu.es/aaveterinaros/aaveterinarios.htm

4. *Reproductive physiology* Reproductive physiology studies and associated technologies increase the success rate of any captive-breeding programme and are important in helping with the conservation of wild felids in captivity. Reproductive technologies are available for three major purposes: (1) assessing fertility and monitoring reproductive status; (2) assisting in breeding and maintenance of gene diversity; (3) learning more about reproductive mechanisms of the Critically Endangered Iberian lynx.

An important outcome of Iberian lynx reproductive physiology studies is the development of non-invasive techniques that aid in captive population management. Over the past 2 years, work carried out to define Iberian lynx ♂ and ♀ hormonal profiles has helped us gain a clearer perspective on the length of breeding periods, and the potential use of hormonal metabolites in faeces as a non-invasive gestation predictor (Jewgenow *et al*., 2006; Pelican *et al*., 2006; see also Schwarzenberger, 2007). While faecal hormones do not seem to be the best diagnostic tool, they have proven to be extremely useful to understand better the year-round reproductive activity of ♂ and ♀ Iberian lynx. Another gestation diagnostic technique, based on analyses of relaxin in urine, is now being researched at the Institute for Zoo and Wildlife Research in Berlin (Jewgenow *et al*., 2006). The non-invasive techniques described above provide a great deal of information, while minimizing the disturbance of the animals under study. Assessment of reproductive health by trans-rectal ultrasound has also been applied to study physiological changes in ♂ and ♀ Iberian lynx (F. Goeritz, unpubl. data). This knowledge has been essential to assess the reproductive soundness of the captive population of Iberian lynx and it is regularly used to make management decisions regarding the pairings of captive animals.

In order to assist breeding and maintaining gene diversity, the Iberian Lynx *Ex Situ* Program collaborates with the maintenance of a Biological Resources Bank for conservation of biomaterial gathered from wild and captive Iberian lynx populations (León *et al*., 2006; Roldán *et al*., 2006). In order to conserve the maximum possible genetic diversity, samples of ♂ and ♀ gametes, as well as different cells or tissues, are being kept. The conservation of gametes allows us to extend future options without the limitations of space, or the risk of disease transmission. Also, the cryopreservation of gametes and embryos allows for the opportunity of prolonging the possibilities of reproduction for individual animals after their death. The preservation of somatic cells (or undifferentiated germ cells) could give individuals who have died before reaching sexual maturity a reproductive opportunity, or extend the reproductive potential of other individuals.

The Iberian lynx BRB is presently being maintained at two locations: the National Museum of Natural Sciences in Madrid (Roldán *et al*., 2006) and the Miguel Hernández University in Elche, Alicante (León *et al*., 2006). Although the Museum of Natural Sciences specializes principally in reproductive samples and the University MH specializes in multi-potential somatic cells, both banks preserve tissue, blood, serum and other biological materials. The storage of these samples means that materials will be available for future analysis whenever needed, which is a valuable resource for potential retrospective studies.

5. *Re-introduction* As mentioned above, the two current small populations of free-ranging Iberian lynxes are highly vulnerable. Thus, it is imperative to create, as soon as possible, new wild populations (while simultaneously increasing numbers in the existing ones).

Before any re-introduction/translocation, a detailed viability study is required (see *IUCN Guidelines for Re-introductions:* IUCN, 1998). It is important to determine whether the cause or causes that brought the species to extinction in the specific area have been eradicated and, if so, whether there is administrative and local population support for the Program and whether the habitat is prepared to support a viable population of the species. All re-introductions and translocations must be performed using scientific support and the Iberian lynx should be no exception (Palomares, 2006). Such conservation techniques require an interdisciplinary approach, with input from experts in ecology, veterinary medicine, physiology and behavioural sciences, as well as support from socio-political and information sciences. All stages of programme development and implementation must have well-defined protocols that document the objectives, methodology, responsibilities, as well as the accountability of the organizations and individuals involved.

The greater the number of captive-bred lynxes produced and trained to maximize their potential for post-release survival, the lower the number of wild lynxes that must be captured to establish new populations, or to reinforce the existing ones (Simón & Cadenas, 2006). Re-introduction and translocation each pose advantages and disadvantages. A comparative study is required to determine which option, or combination of options, is most appropriate for the conservation of the Iberian lynx.

6. *Communication, awareness and training* Awareness, education and scientific training are essential to all conservation-breeding programmes. Education and awareness efforts should be focused on changing prevalent attitudes that contribute to habitat destruction and species extinction. One advantage enjoyed by conservation-breeding programmes is their ability to gain public attention, particularly if the animal in question is charismatic and attractive to the broader public. The Iberian lynx is one such case and raising public awareness of the need for habitat conservation to guarantee survival of the species in the wild is one of the Program's primary objectives. It is important to emphasize that breeding and keeping lynxes in captivity, with no hope of ever returning them to the wild or of recovering the natural population, would be a pointless exercise. The breeding programme encourages, cooperates with and supports media interest in the Iberian lynx, while taking every opportunity to remind the public of the primary importance of *in situ* conservation work.

We also share on-line information on the Iberian lynx web page (http://www.lynxexsitu.es), featuring monthly newsletters, pictures and videos of all 'captive' lynxes, along with general interest and scientific articles, and descriptions of the Center's protocols and working methods. An English-language version is currently in production, to further expand the scope of communication and awareness efforts. The Web page also contains an area only accessible to managers, researchers and technical personnel working directly with the Program, for restricted database access and other information exchange. As part of its training efforts, the Acebuche Center Breeding Program organizes on-site internships for recent college graduates interested in acquiring first-hand knowledge within an endangered species conservation programme.

CONCLUSION

Besides offering an insurance against extinction, the Iberian Lynx Conservation Breeding Program emphasizes how results from multidisciplinary life-science research can be integrated into an adaptive management approach to help recover the world's most threatened felid species. The ultimate goal of the *ex situ* Program is to offer sound support to *in situ* conservation efforts by providing healthy Iberian lynx for future re-introduction

projects. In addition, the Program carries out a communication plan aimed at preparing professionals for working with threatened-species conservation as well as sensitizing the general public and decision-makers about the importance of conserving habitat for the recovery of this charismatic felid. An effective recovery of the Iberian lynx in nature will help protect large areas of Mediterranean forests and scrubland, thus benefiting a wide variety of species that thrive on this unique and important ecosystem.

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